Medical Staff Conference

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Insulin Receptors: New Insights Into Old Problems

These discussions are selected from the weekly staff conferences in the Department of Medicine, University of California, San Francisco. Taken from transcriptions, they are prepared by Drs. David W. Martin, Jr., Associate Professor of Medicine, and Robert C. Siegel, Associate Professor of Medicine and Orthopaedic Surgery, under the direction of Dr. Lloyd H. Smith, Jr., Professor of Medicine and Chairman of the Department of Medicine. Requests for reprints should be sent to the Department of Medicine, University of California, San Francisco, CA 94143.

DR. SMITH:* *Diabetes mellitus is perhaps the most important metabolic disease in the United States today. The discovery of insulin more than 50 years ago gave initial promise that the disease was at least explained pathogenetically and that the ultimate therapeutic tool was at hand. As we all know both of these expectations have been shown to be premature. It is remarkable that a half century of research still leaves insulin as clarified in structure but obscure in function, at least at the molecular level.*

Hormones must function by interaction with cells. Attention has recently been turned to the events of that surface interaction. Dr. Ira Goldfine has been an active investigator in this field and will review for us the current status of our understanding of insulin receptors.

DR. GOLDFINE:† Today, I will talk about an exciting new area of insulin action and diabetes mellitus, the recent direct studies of the binding of insulin to receptors in target tissues. As you are undoubtedly aware, significant progress has been made in understanding the biochemical mechanisms involved in certain states of primary and secondary diabetes. I believe, however, that it is appropriate at this point to give a few words of caution concerning studies in this field. It has been said that searching for the cause of diabetes mellitus is somewhat like the peeling of an onion, not only can the process bring tears to the eyes, but after each successful step, one is still left with the layer beneath. With this in mind, I will first discuss theoretical mechanisms of insulin action. Next, I will talk about the status of insulin receptors in states of primary and secondary diabetes. Finally, I will say a few words about possible future studies.

The Mechanism of Action of Insulin

Insulin is a relatively small protein comprised of 51 amino acids in two polypeptide chains, an A chain and a B chain, connected by two disulfide

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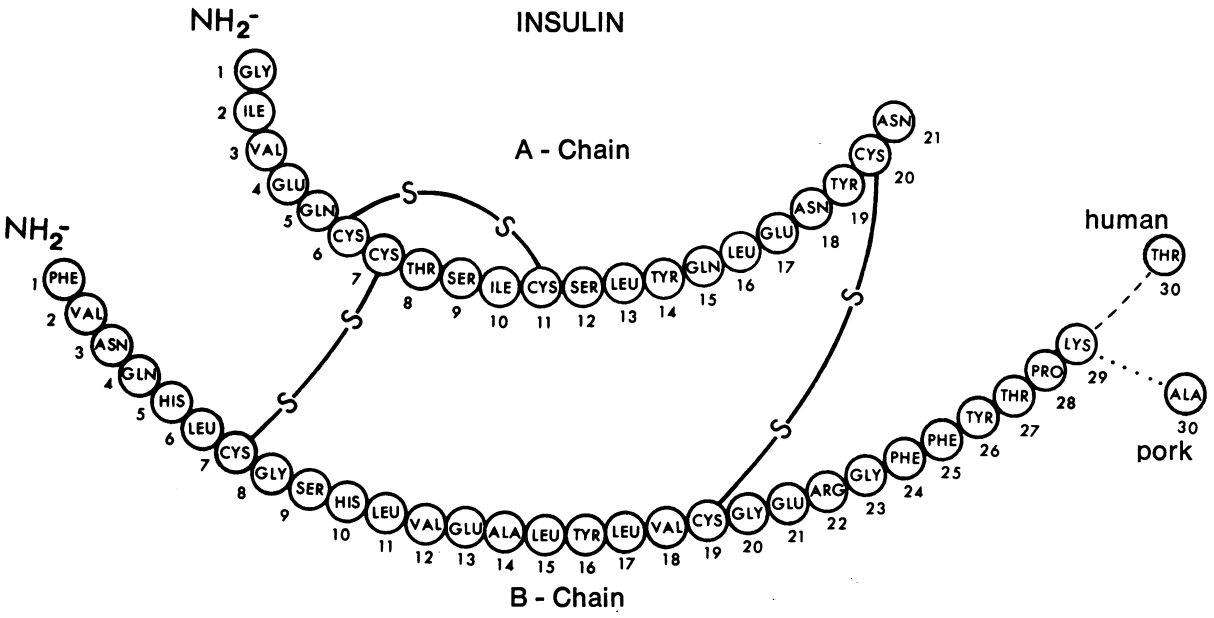

Figure 1.—The amino acid sequences of human and pork insulins. Note that they differ by only one amino acid (i.e., B-30).

bridges (Figure 1).[1-2] The three dimensional structure of insulin has been elucidated by Dr. Dorothy Hodgkin and co-workers via x-ray crystallography, and with that type of analysis, insulin appears to be a globular protein containing an alpha helix, an antiparallel B pleated sheet, a hydrophobic interior, and a hydrophilic exterior.

The pronounced hormonal potency of insulin in many tissues results from its ability to regulate a wide variety of cellular processes.[1-3] The net result of insulin's actions is both to promote the storage and utilization of metabolic substrates, and to increase the synthesis of macromolecules. Insulin is capable of exerting these anabolic effects because it is able to influence cellular functions at a variety of subcellular levels. For instance, insulin increases transport at the cell membrane; activates or inhibits enzymes in the cytosol, mitochondria and endoplasmic reticulum; stimulates new protein synthesis in the rough endoplasmic reticulum, and regulates deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis in the cell nucleus (Table 1). The question that arises, however, is how can a small protein like insulin carry out such a wide variety of diverse effects in target tissues.

At present there are two theories of insulin action, as shown in Figure 2. The first theory, the second-messenger theory of insulin action,[1,4] is based by analogy on the action of hormones such as glucagon. Glucagon acts via a second messenger, cyclic adenosine monophosphate (cyclic AMP), that is generated at the plasma membrane and in turn carries out many, if not all, of the intracellular effects of glucagon. For a number of years, investigators have postulated that a similar type of second messenger exists for insulin. For instance, Ca^{+2} and cyclic guanosine monophosphate (cyclic GMP) have been considered as candidates for this role. At present, however, no unique second messenger for insulin has been discovered.[3] The other theory, favored in our laboratory, is the direct intracellular binding theory of insulin action.[3,5] This theory is based on two recent observations. The first is that specific binding sites for insulin are present not only on the plasma membrane of target cells, but also on intracellular organelles, such as nuclei, smooth and rough endoplasmic reticulum, and Golgi membranes.[3-5] The second observation is that insulin can enter target cells and bind to these sites.[5] Therefore, it is possible that insulin directly regulates functions at a variety of cellular levels and a second messenger is not needed. Further studies will be necessary to discern which of the two theories is the correct one.

Measurement of Hormone Receptors

The modern era of measuring hormone receptors began in 1970. Before this, investigators had employed radiolabeled hormones to measure hormone binding sites on target tissues, but these

TABLE 1.—*Actions of Insulin at Various Subcellular Levels*[1-3]

Cell membrane
Stimulation of transport: sugars, amino acids, fatty acids, ions and nucleic acid precursors
Activation and inhibition of enzymes
Change of membrane potential
Alterations of cell surface morphology
Cytosol
Activation and inhibition of enzymes
Endoplasmic reticulum
Activation and inhibition of enzymes
Ribosome
Increased protein synthesis
Mitochondria
Activation of enzymes
Lysosome
Inhibition of protein degradation
Nucleus
Modulation of DNA and RNA synthesis

studies were plagued with two problems. The first problem was whether radiolabeled hormones (usually one made radioactive by substituting one or more iodine atoms into tyrosine residues) were biologically active. The second was whether the binding observed represented the interaction of the hormone with a biologically important binding site, that is, a receptor. Both these problems were simultaneously overcome in the laboratory of Dr. Jesse Roth, who in collaboration with Dr. Robert Lefkowitz and others prepared biologically active iodinated adrenocorticotropic hormone (ACTH) and showed its binding to specific receptors in adrenal tissue.[6] Soon afterwards Dr. Roth, in collaboration with Dr. Pierre Freychet, carried out similar studies for insulin.[7] Subsequently, a large number of laboratories began to measure the binding of insulin to receptors in target tissues.

The methodology employed for measuring polypeptide hormone binding to receptors (radioreceptor assay) follows the same basic techniques as devised by Drs. Solomon Berson and Rosalyn Yalow for the radioimmunoassay of polypeptide hormones. In radioreceptor assays, hormones are first radiolabeled (usually either with iodine 125 or iodine 131) under conditions where the biological activity of the hormone is preserved. Next, either isolated whole cells or purified subcellular fractions are employed and the binding of the hormone to receptors is measured.

Radioreceptor Assay of Insulin

Currently, we are able to measure insulin receptors in a wide variety of tissues and cell fractions. In particular, isolated lymphocytes, fat cells

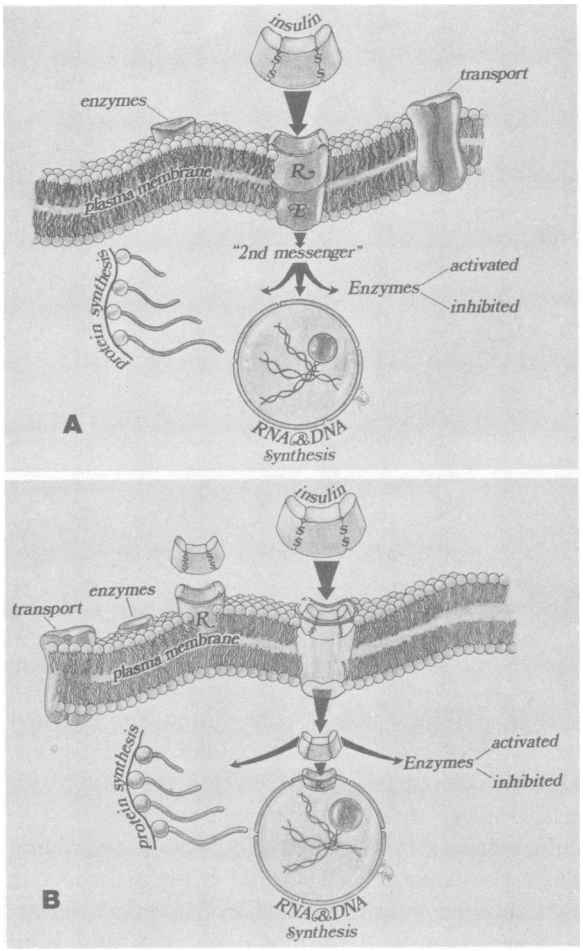

Figure 2.—Theories of insulin action. **A**, second messenger theory of insulin action. **B**, intracellular binding theory of insulin action.

and liver cells are commonly used. One interesting aspect of this line of research is that the insulin receptor is very similar in all tissues from a variety of species, and, therefore, a characteristic of an insulin receptor seen in one tissue usually applies to receptors on other tissues.[4-8] The insulin receptor appears to be a protein with four subunits and the entire molecule has a molecular weight of approximately 300,000 daltons.[2,4,8] This receptor has a high affinity for insulin (Kd 10^{-8} to 10^{-10}M) and specifically binds only biologically active insulins or insulin derivatives.[2,4,8] The most widely characterized subcellular fraction where insulin receptors have been studied is the plasma membrane (cell surface membrane) of a variety of isolated cells. In addition, we can measure specific insulin binding sites in smooth and rough endoplasmic reticulum, Golgi membranes and nuclear membranes.[3-5] Interestingly, the receptors

TABLE 2.—*States of Insulin Sensitivity and Resistance That Correlate with Receptor Defects*

	Receptors	*Plasma Insulin*
Sensitivity		
Hypophysectomy	Increased	Decreased
Adrenalectomy	Increased	Decreased
Resistance		
Obesity	Decreased	Increased
Glucocorticoid Excess	Decreased	Increased
Diabetes Mellitus:		
With Hyperinsulinemia	Decreased	Increased
With Acanthosis Nigricans	Decreased	Increased
With Lipodystrophy	Decreased	Increased

TABLE 3.—*States of Insulin Resistance That Suggest Postreceptor Defects*

	Receptors	*Plasma Insulin*
Growth Hormone Excess	Unchanged	Increased
Aging	Unchanged	Increased
Fasting	Increased	Decreased

TABLE 4.—*Decreased Binding of Insulin to Circulating Monocytes from Obese Humans**

	Percent Insulin Bound (10^5 cells /ml)
Thin Controls	6.0±1.1
Obese	2.8±0.3

In these studies monocytes were isolated from the blood of both normal thin controls and obese subjects. These cells were then incubated with radioiodinated insulin and the specific binding measured.

*Adapted from Roth, et al.[8]

on these intracellular organelles have certain binding characteristics that are similar to those found on plasma membranes.

States of Altered Sensitivity to Insulin

It is known that the sensitivity of target tissues to hormones, such as insulin, can be altered at several levels. These levels can be classified as prereceptor, receptor and postreceptor. A classic example of prereceptor insulin resistance is the case of an insulin-requiring diabetic patient who has very high circulating levels of anti-insulin antibodies. Soon after the ability to measure insulin receptors in target tissues became available, investigators then looked at the status of the insulin receptor in a variety of states of altered sensitivity to insulin to determine whether receptor or postreceptor changes exist (Tables 2 and 3). Today, I will primarily discuss states of altered sensitivity to insulin that result from alterations in the insulin receptor. Two well-known clinical states of insulin resistance associated with receptor

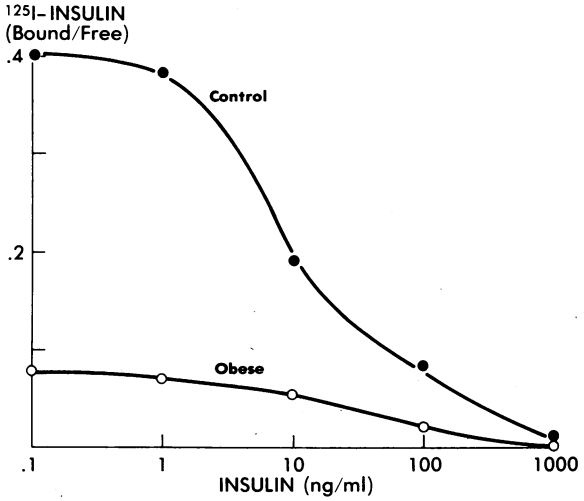

Figure 3.—Decreased binding of insulin to liver plasma membranes from obese hyperglycemic mice when compared with normal thin litter mates. For convenience, in these and subsequent figures, the radioreceptor binding data will be plotted in a manner similar to that commonly employed for radioimmunoassay; that is, the ratio of bound over free labeled insulin as a function of the logarithm of the insulin concentration in the binding reaction. This type of plot reflects both the affinity and capacity of the binding site. (Adapted from Kahn[4] and Roth, et al.[8])

changes are those associated with obesity and glucocorticoid excess. Two classic states of enhanced insulin sensitivity which appear to be related to receptor changes are those that occur following hypophysectomy and adrenalectomy. In addition, hyperinsulinemic diabetes is considered by some investigators to be an insulin resistant condition.

States of Receptor Alterations

The first state of insulin resistance to be investigated intensively was that of obesity. Initial studies were done with an animal model of pronounced obesity, obese hyperglycemic mice.[4,8] These are animals with a pair of recessive genes that cause massive obesity. They are hyperglycemic despite 10- to 100-fold elevations of plasma insulin levels. When plasma membranes from liver or fat are prepared from these animals, and compared with either normal animals or normal litter mates, the binding of insulin is notably diminished (Figure 3). If these obese animals are fasted to reduce body weight, plasma insulin levels decrease and the diminished binding tends to return to normal. The same features are seen in humans with acquired obesity (Table 4).[8] When insulin receptors on their circulating blood monocytes

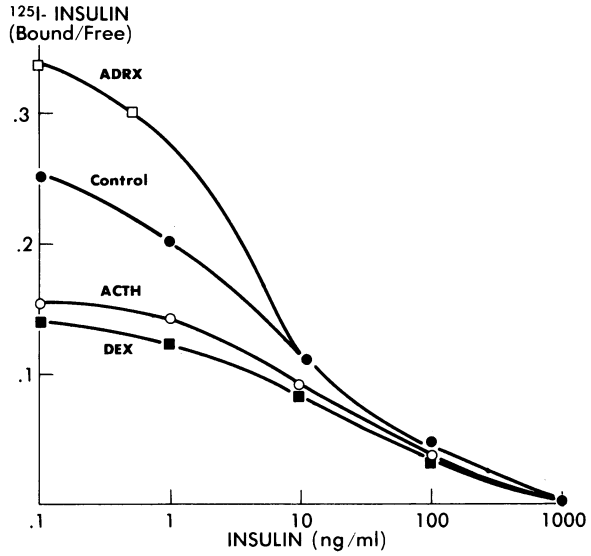

Figure 4.—Binding of insulin to liver plasma membranes from control, adrenalectomized (ADRX), adrenocorticotropic hormone (ACTH) treated and dexamethasone treated (DEX) rats. I. D. Goldfine and C. R. Kahn (unpublished observations).

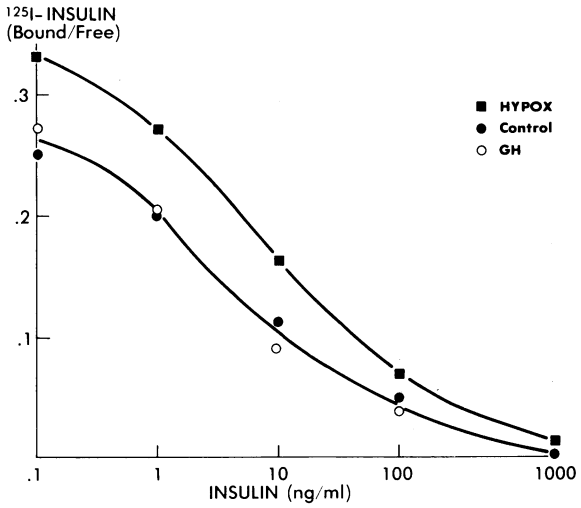

Figure 5.—Binding of insulin to liver plasma membranes from control, growth hormone treated (GH), and hypophysectomized rats (HYPOX). I. D. Goldfine and C. R. Kahn (unpublished observations).

are measured, it can be seen that cells from obese patients have a greatly diminished ability to bind insulin.

Another instance of severe insulin resistance is that which can occur during states of glucocorticoid excess. Although corticosteroids can rapidly antagonize certain effects of insulin—such as those effects on glucose transport—without influencing insulin receptors, it recently has been observed that either steroid or ACTH treatment of animals for several days is associated with a decrease in the binding of insulin to receptors in liver and fat (Figure 4). Therefore, chronic hyperglucocorticoidism is another clinical state of insulin resistance, where the insulin receptor appears to play a role in the development of this clinical problem.

There is a unique syndrome of extreme insulin resistance associated with acanthosis nigricans and various immunological problems.[9] In one group of these rare patients, a circulating antibody to the insulin receptor can be detected. This antibody competitively blocks the binding of insulin to target tissues, and it is believed that the presence of this antibody accounts for the insulin resistance observed. In lipodystrophy, there is insulin resistance, and it recently has been observed that patients with this problem have a diminished number of insulin binding sites on their circulating monocytes.[10]

We will next consider two insulin sensitive states, that following hypophysectomy and that following adrenalectomy. In both instances, plasma membranes from animals with these conditions bind more insulin than membranes from control animals (Figures 4 and 5). Consequently, it is possible that the increased number of receptors seen in these two states may account for their increased sensitivity to both endogenous and exogenous insulin.

Insulin Receptor Regulation by Insulin Itself

Before discussing the status of the insulin receptor in diabetes mellitus, I wish to mention important new data concerning the regulation of the insulin receptor by insulin itself. When certain insulin sensitive and insulin resistant states are examined, it becomes apparent that an inverse correlation exists between the insulin levels in the plasma and the number of insulin receptors in target cells (Table 2). Therefore, in certain states of insulin resistance where there are high insulin levels, such as obesity or glucocorticoid excess, the number of insulin receptors are decreased. Inversely, in certain states of insulin sensitivity, when insulin levels are decreased, such as after hypophysectomy or adrenalectomy, the number of receptors is increased. Drs. Jesse Roth and James Gavin, and their colleagues, undertook studies to determine if there is a direct correlation between these two factors.[8] To determine whether an effect of insulin was mediated directly

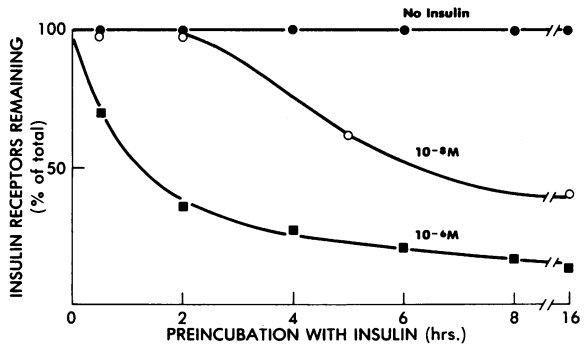

Figure 6.—*In vitro* demonstration that insulin itself can regulate its own receptor. (Adapted from Roth, et al.[8])

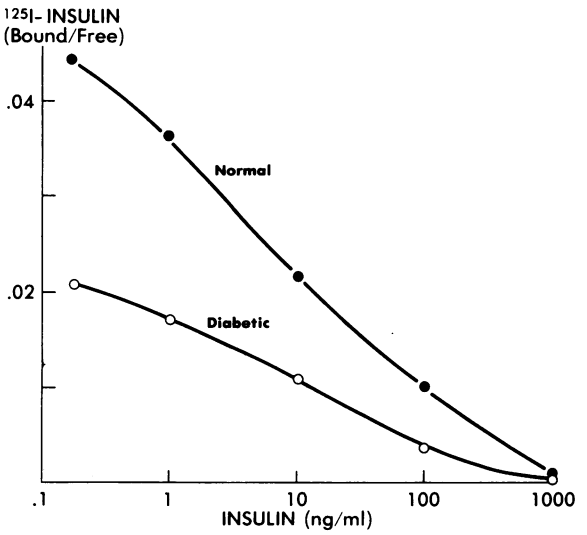

Figure 7.—Binding of insulin to monocytes from normal and diabetic subjects. (Adapted from Olefsky and Reaven.[11])

by insulin and not indirectly through changes in other hormones or metabolites, they employed an *in vitro* system, isolated human cultured lymphocytes. These cells, which are maintained in permanent culture, have large numbers of insulin receptors that are virtually identical to those seen on classical target tissues for insulin, such as liver and fat. When they preincubated these cells with unlabeled insulin for varying periods, and then extensively washed the cells to remove any insulin bound to the surface, they found that the subsequent binding of radiolabeled insulin was diminished (Figure 6). When they employed high concentrations of insulin, such as 10^{-6}M, they found that after periods as short as 30 minutes a significant fall in the insulin receptors could be detected. At this concentration, greater than 75 percent of the receptors were lost after

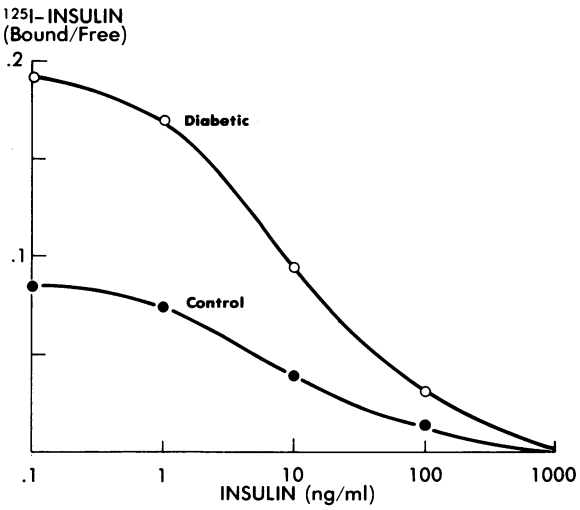

Figure 8.—Binding of insulin to liver plasma membranes from control and streptozotacin-diabetic rats. (Adapted from Davidson and Kaplan.[12])

eight hours of incubation. Similar effects, smaller in magnitude and slower in onset, were seen with more physiological concentrations of insulin,* 10^{-8}M. They postulated that insulin autoregulated its own receptor in an inverse fashion and then made the interpolation of these *in vitro* experiments to *in vivo* studies. It is now apparent that, *in vivo,* high concentrations of insulin will lower the number of insulin receptors on a number of target tissues. This observation provides a partial explanation for the changes in number of receptors noted in the aforementioned studies.

Insulin Receptors in Diabetes Mellitus

There has been a controversy for a number of years concerning whether insulin resistance exists in diabetes mellitus. There appear to be convincing data that in patients with mild to moderate diabetes who have higher than normal levels of insulin, there is insulin resistance. This subject has been extensively studied in the laboratories of Drs. Gerald Reaven and Jerrold Olefsky.[11] When the binding of insulin to circulating peripheral monocytes from such diabetic patients is examined, cells from these subjects bind approximately half as much insulin as do similar tissues from control subjects (Figure 7). In contrast, when animals with experimental diabetes are studied, a different picture comes forth. In diabetic animals studied by Drs. Mayer Davidson and Solomon Kaplan[12] there is an increase in the

*The basal fasting concentration of plasma insulin in man is approximately 10^{-10}M (0.6 ng/ml or 15 μU/ml). After stimulation this level can rise 10- to 100-fold.

TABLE 5.—*Plasma Insulin Levels and Glucose Values in Diabetic Humans and Rats*

	Glucose (mg/dl)	*Insulin (μU/ml)*
Humans[11]		
Controls	89±6	11±1
Diabetics	203±18	21±2
Rats[12]		
Controls	145±2	26±2
Diabetics	515±12	7±1

binding of insulin to target tissues when compared to controls (Figure 8). The apparent discrepancy between the findings in humans and those in animals can best be understood when the insulin levels in the patients and animals are examined (Table 5). In these diabetic humans the insulin levels are higher than normal and, therefore, in light of the previous data concerning autoregulation of insulin receptors it is not surprising that their receptor concentrations are diminished. In contrast, the insulin levels in the diabetic animals are decreased, and therefore, it is also not surprising that their receptor levels are increased. Therefore, it appears that in diabetes there can be either an increase or decrease in insulin receptors, depending on the plasma insulin level.

States of Postreceptor Alterations

There are several conditions in which the insulin receptor does not appear to play a major role in the altered response of target tissues to insulin and the altered responses, therefore, are subsequent to receptor binding. One such state is that of growth hormone excess or acromegaly. In certain studies, up to 25 percent of acromegalics have been found to be either diabetic or have notably abnormal glucose tolerance tests. When rats are injected with growth hormone, however, there is very little difference in the binding of insulin to plasma membranes despite elevated levels of plasma insulin (Figure 5). Therefore, states of growth hormone excess, in contrast to obesity and glucocorticoid excess, may not be associated with major alterations in the binding of insulin (Table 3).

Another condition associated with postreceptor alterations is fasting, where insulin receptors are increased but insulin resistance is present. A third postreceptor condition is aging, an insulin resistant state where receptors are unchanged in liver and lymphocytes, but where the response of these tissues to insulin is decreased.[13]

Future Directions

The studies I have discussed indicate that insulin receptors, and especially the receptors present on the plasma membrane of target cells, are altered in several states of either insulin sensitivity or insulin resistance. Although the metabolic changes that occur in these various states are complex, it would appear that changes in the insulin receptor in certain conditions contributes to the abnormalities observed. Many important questions, however, remain unanswered.

One question is how are the cell-surface receptors for insulin regulated by insulin itself. One possibility is that insulin influences target cells to decrease receptor synthesis. Another is that insulin may irreversibly alter the conformation of its receptor so that the receptor loses its binding capabilities. A third possibility, which I believe is the most intriguing, is that after binding to its cell surface receptor, both insulin and its receptor enter the cell together. The receptor could be degraded while insulin then binds to intracellular binding sites.

Since plasma membrane binding sites are altered by insulin, the question arises whether in the same conditions intracellular binding sites for insulin change in concert with those on the cell surface. Recent studies by Dr. Riccardo Vigneri and myself indicate that the intracellular binding sites on nuclei and on smooth and rough endoplasmic reticulum are also regulated by insulin itself in a manner very similar to that seen for those sites on the plasma membrane.

From a clinical point of view, the question arises as to the importance of the regulation of the insulin receptor by insulin itself during various treatment regimens. For instance, it has been noted that in the treatment of diabetic ketoacidosis, the administration of very small amounts of insulin can lower sugar and ketone levels in a manner comparable to that obtained when very large amounts of insulin are employed. On the basis of the aforementioned data, it is possible that by giving very large amounts of insulin there can be a rapid loss of insulin receptors induced by insulin itself. This process, in turn, would increase the need for more insulin, setting up a vicious cycle. It would seem that one of the potential benefits of using smaller amounts of insulin for treating diabetic ketoacidosis would be to avoid this phenomenon. Another similar clinical problem could occur in the chronic treat-

ment of patients with diabetes mellitus. It would appear possible that by administering excessive amounts of insulin, a reduction in the number of receptors would occur which, in turn, would continue the need for large doses of insulin.

It appears, therefore, that a new era in diabetes has opened with the ability to measure the insulin receptor in target tissues. These studies have been applied in various forms of primary and secondary diabetes and exciting new data have been obtained. I believe, therefore, that in the next few years much more data will come forth to help us understand the biochemical mechanisms underlying these conditions.

REFERENCES

1. Pilkis SJ, Park CR: Mechanism of action of insulin. Ann Rev Pharmacol 14:368-385, 1974

2. Steiner DF: Insulin today. Diabetes 26:322-340, 1977

3. Goldfine ID: Does insulin need a second messenger. Diabetes 26:148-154, 1977

4. Kahn CR: Membrane receptors for hormones and neurotransmitters. J Cell Biol 70:261-286, 1976

5. Goldfine ID, Smith GJ, Wong KY, et al: Cellular uptake and nuclear binding of insulin in human cultured lymphocytes: Evidence for potential intracellular sites of insulin action. Proc Natl Acad Sci USA 74:1368-1372, 1977

6. Lefkowitz RJ, Roth J, Pricer W, et al: ACTH receptors in the adrenal: Specific binding of ACTH-^{125}I and its relation to adenyl cyclase. Proc Natl Acad Sci USA 65:745-752, 1970

7. Freychet P, Roth J, Neville DM Jr: Insulin receptors in the liver: Specific binding of [^{125}I] insulin to the plasma membrane and its relation to insulin bioactivity. Proc Natl Acad Sci USA 68:1833-1837, 1971

8. Roth J, Kahn CR, Lesniak MA, et al: Receptors for insulin, NSILA-s, and growth hormone: Applications to disease states in man. Recent Prog Horm Res 31:95-139, 1975

9. Flier JS, Kahn CR, Roth J, et al: Antibodies that impair insulin receptor binding in an unusual diabetic syndrome with severe insulin resistance. Science 190:63-65, 1975

10. Oseid S, Beck-Nielsen H, Pedersen O, et al: Decreased binding of insulin to its receptor in patients with congenital generalized lipodystrophy. N Engl J Med 296:245-248, Feb 3, 1977

11. Olefsky JM, Reaven GM: Decreased insulin binding to lymphocytes from diabetic subjects. J Clin Invest 54:1323-1328, 1974

12. Davidson MB, Kaplan SA: Increased insulin binding by hepatic plasma membranes from diabetic rats. J Clin Invest 59: 22-30, 1977

13.Goldfine ID: Binding of insulin to thymocytes from suckling and hypophysectomized rats: Evidence for two mechanisms regulating insulin sensitivity. Endocrinology 97:948-954, 1975

The Surgeon Who is a Bacterial Shedder

If you knew you were a shedder, what would you do?

I would first decide where I was shedding, and there are a number of places where you can shed: you can shed from the skin (generally, that's few and far between); you can shed from the nasopharynx (that's, I think, the most common source of shedders); you can shed from axilla or the groin or you can shed from the perineum. People tend not to . . . shed in all places at once; they seem to shed from one place or another. And then you go to work on the area that's causing the trouble. Experience says that by using topical antibiotics aimed specifically at the bacteria that's causing the problem (and it's usually a staphylococcus aureus of one type or another), the sources of the bacterial growth on the surface can be controlled and the shedding eliminated. Now, this probably is going to be a recurrent problem, because you can wipe it out for a little while and then you are going to start regrowing it again, so I think the person who is a shedder has to be continually alert to the problem. But I think that it's not an impossibility. You don't have to go into dermatology or x-ray or some other fancy specialty. You can continue in surgery safely, I think, if you pay attention to the problem and deal with it sensibly.

—JOHN F. BURKE, MD, *Boston*

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